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Capsular Antibodies Induce Type-Specific Phagocytosis of Capsulated *Staphylococcus aureus* by Human Polymorphonuclear Leukocytes

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Capsular types 5 and 8, which account for about 70% of *Staphylococcus aureus* strains isolated from the blood of patients, resisted in vitro phagocytosis by human polymorphonuclear leukocytes (PMN). Antisera and monoclonal antibody to type 5 and 8 capsular polysaccharides (CPS) induced type-specific in vitro phagocytosis of capsulated organisms by PMN. Antibodies directed against the *O*-acetyl moiety of the type 8 CPS were more effective in inducing phagocytosis of type 8 organisms by PMN. Either type-specific antiserum or monoclonal antibody reactive with the native O-acetylated type 8 CPS was most effective in inducing in vitro phagocytosis of type 8 organisms by PMN. These results provide further evidence that CPS of *S. aureus* are associated with host immunity to this organism.

Eleven antigenically distinct capsular polysaccharides (CPS) are recognized for *Staphylococcus aureus* (2, 5, 7, 11, 12, 14, 19). Two of these, type 5 and type 8, comprise about 70% of the isolates from patients with *S. aureus* disease and from human carriers (2, 8, 11, 22). Type 5 and 8 CPS were visualized on the surface of *S. aureus* by electron microscopy (8, 22). Both types of CPS contain the trisaccharide repeat unit ManNAcA-FucNAc-FucNAc. However, the repeat units of the two polysaccharides differ in glycosidic linkages (14).

S. aureus bacteremia usually occurs in patients with decreased immunologic resistance (20). The virulence mechanism(s) involved in invasive disease caused by *S. aureus* have not been satisfactorily defined. It has been proposed that phagocytosis followed by intracellular killing of *S. aureus* by polymorphonuclear leukocytes (PMN) is an important host resistance mechanism (3, 13, 26, 27). This mechanism is mediated by antibody to CPS and complement (26, 27). Capsulated strains are more resistant to phagocytosis by PMN than are noncapsulated *S. aureus* strains (13, 26, 27). These studies, however, either utilized strains of *S. aureus* with CPS types not associated with bacteremic disease or employed antibodies of undefined specificities.

We report that polyclonal antibody or monoclonal antibody (MAb), specific for type 5 and 8 CPS induces in vitro phagocytosis of *S. aureus* strains from patients with bacteremia. Antibodies specific for the epitope containing the *O*-acetyl group of the "native" type 8 CPS were most efficient in inducing the phagocytosis of capsulated type 8 organisms by PMN.

MATERIALS AND METHODS

Bacterial strains. The strains of *S. aureus*, their sources, and CPS types are listed in Table 1.

Antisera. Rabbits were immunized with formalinized *S. aureus* prepared as described previously (2, 11). Blood was drawn 4 days after the last injection, and the serum was collected by centrifugation under sterile conditions and stored at −4°C. Noncapsulated *S. aureus* 1 was used to prepare rabbit anti-teichoic acid antiserum. Production of murine (BALB/c) hybridomas was performed by a modification of the method of Kohler and Milstein (17). The hybridomas were screened by enzyme-linked immunosorbent assay as described earlier and exhibited greatest reactivity with immunoglobulin M-specific reagents (6, 24).

***S. aureus* CPS.** Type 8 CPS were prepared from strains Wright and I-58, and type 5 CPS was prepared from strain Lowenstein as described previously (5). These preparations contained less than 2% protein or nucleic acids by weight. De-O-acetylated type 8 CPS were prepared by treating CPS with 0.1 M NaOH at 37°C for 4 h.

In vitro phagocytosis. PMN were isolated from the peripheral blood of a single donor and suspended in RPMI medium (Microbiological Associates, Bethesda, Md.) supplemented with 5% heat-inactivated fetal calf serum to give a cell count of 10^7 cells per ml (15, 16, 21). *S. aureus* strains were grown in Columbia broth (Difco Laboratories, Inc., Detroit, Mich.) for 8 h at 37°C and centrifuged. The packed cells were adjusted to 10^7 cells per ml with RPMI medium. The incubation mixture contained 0.5 ml of a PMN suspension (5×10^6 cells), 0.1 ml of antiserum or MAb, and 0.1 ml of a bacterial suspension (10^6 cells). RPMI medium was added to each mixture to bring the volume to 1.0 ml. Controls included bacteria incubated with antiserum alone and PMN incubated without antiserum. Samples of each mixture (100 μl) were diluted into 9.9 ml of distilled water at 0, 60, and 120 min to disrupt the PMN. A twofold dilution series of each lysate was prepared, and 100-μl portions were plated onto Columbia agar plates (Difco) in triplicate. T_0 values given in all tests represent the observed cell counts on Columbia agar. The results of viable cell counts are the means of three experiments with PMN from a single donor. Other human and bovine PMN were also effective; however, the donor mentioned above was a more available source.

Microscopy. Bacteria and PMN were prepared for light microscopy by staining with Giemsa. Bacteria were incubated with PMN for 1 h as described above. The incubation mixture was centrifuged at 1,000 rpm, and the cell pellet was

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TABLE 1. *S. aureus* strains

Strain	Source[a]	Capsular type
E 2286	SSI	8
Becker	Kaiser	8
Wright	Kaiser	8
I-58	Assaf	8
83	Penn State	8
I	Assaf	8 variant
Lowenstein	Kaiser	5
E-5975	SSI	5
E-6657	SSI	5
170	Assaf	NT[b]
1	Assaf	NT

[a] SSI, Vibeke Thamdrup Rosdahl, Staphylokok Laborotariet, Statens Serum Institut, Copenhagen, Denmark; Kaiser, Kaiser Permanente Hospital, Hollywood, Calif.; Assaf, Assaf Harofeh Hospital Medical Center, Zerifin, Israel; Penn State, Pennsylvania State University, University Park, Pa.

[b] NT, Nontypable.

smeared on a microscopy slide. The specimens were air dried, heat fixed, and then stained with Giemsa for 1 min and washed with distilled water.

Serologic methods. CPS antibodies were measured by quantitative precipitin analysis (10, 12). The amount of precipitated antibodies was assayed by a modification of the Lowry method (10). Double immunodiffusion was performed in 0.7% agarose (BBL Microbiology Systems, Cockeysville, Md.), in 0.05 M phosphate-buffered saline (pH 7.4), and with CPS at 0.1 mg/ml.

RESULTS

Phagocytosis of capsulated *S. aureus*. Capsulated strains of *S. aureus* types 5 and 8 resisted killing by PMN in the presence of normal rabbit serum (Fig. 1). The viable cell counts of the capsulated strains did not decrease during the 120 min of incubation. Noncapsulated strains 170 and 1, in contrast, were rapidly killed, as indicated by a decline in their viable cell counts within 120 min. Figure 2 shows that type 8 CPS antisera prepared against two different type 8 strains induce killing of the type 8 organism E-2286 by PMN. Normal rabbit serum or anti-teichoic acid serum prepared in rabbits injected with noncapsulated strain 1 did not induce phagocytosis of *S. aureus* by PMN. On the basis of previous studies, we interpret PMN and antibody-dependent killing of *S. aureus* as phagocytosis (13, 26, 27).

The induction of PMN phagocytosis of type 8 and type 5 *S. aureus* by anti-CPS antibodies was type specific. Phagocytosis of capsulated type 8, strain E-2286 by PMN was observed only with type 8 antiserum (anti-strain 83) (Fig. 3). The type 5 antiserum was ineffective. Experiments in which rabbit antisera to *S. aureus* CPS types 1-4, 6, and 7 failed to induce phagocytosis of either of the type 8 organisms by PMN are not shown. Similarly, type 5, strain E-5975 was phagocytized by PMN in the presence of type 5 antiserum but not in the presence of anti-type 8 serum (Fig. 3).

Further proof that anticapsular antibody mediates in vitro phagocytosis of encapsulated *S. aureus* was provided by the utilization of type 8 monoclonal antibodies in this assay (Fig. 4). Fig. 4A shows that only a few type 8 organisms were associated with PMN in the presence of anti-teichoic acid serum. This antiserum was derived from a rabbit injected with strain 1, a noncapsulated *S. aureus* strain. In contrast (Fig. 4B), most of the type 8 organisms were associated with PMN in the presence of an immunoglobulin M type 8 antibody preparation, MAb 1725 (reactive with only native O-acetylated CPS), and were not found free in the medium.

The specificity of the type 8 serum participating in the in vitro phagocytosis reaction was studied with two type 8 antisera and MAbs of different specificities reacting with the native O-acetylated and the de-O-acetylated polysaccharides. The specificity of two type 8 antisera is illustrated in Fig. 5 by quantitative precipitin analyses. Antiserum Becker, the prototype 8 CPS antiserum, reacted with native O-acetylated CPS (Fig. 5); only slight precipitin formation was observed with the de-O-acetylated (NaOH-treated) type 8 CPS. In contrast, antiserum against strain I with both O-acetylated and de-O-acetylated CPS (Fig. 5), suggesting the presence of two populations of antibodies. Antibodies in the anti-strain I serum reactive with O-acetylated or de-O-acetylated polysaccharide could be selectively removed by adsorption with type 8 bacteria before and after NaOH treatment. The effectiveness of the adsorption was confirmed by quantitative precipitation (data not shown).

These two selected antisera (strains Becker and I) and MAbs were used in the in vitro phagocytosis test. A list of the antisera, MAbs, and their relative titers is given in Tables 2 and 3. Figure 6 shows the results of a phagocytosis experiment using the selected type 8 rabbit antiserum and MAbs of various specificities (24, 25). MAb 1725, which is

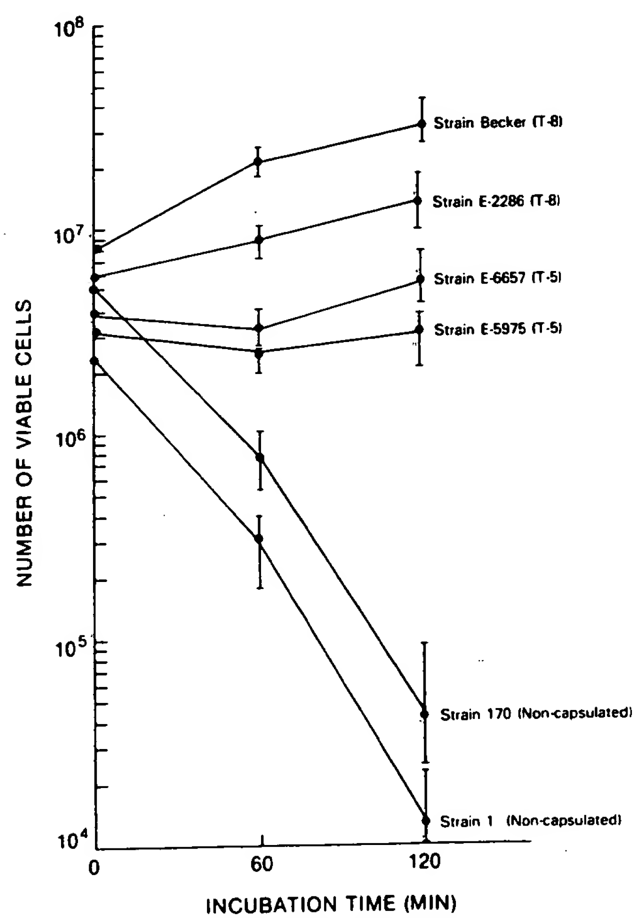

FIG. 1. Resistance of capsulated strains (Table 1) of *S. aureus* to in vitro phagocytosis by PMN in the presence of normal rabbit serum. Noncapsulated control strains were strains 170 and 1. *S. aureus* strains Becker, E-2286, E-6657, and E-5975 were suspended in RPMI buffer, mixed with 0.1 ml of antiserum dilution and 0.5 ml of human PMN preparation, and incubated for various times at 37°C. Samples were collected and plated onto Columbia agar. Colonies of *S. aureus* were counted and recorded.

TABLE 2. *S. aureus* antisera and CPS type specificities

S. aureus	CPS type specificity	CPS antibody (mg/ml)[a]
E-2286	Type 8	8.0
83	Type 8	12.0
Becker	Type 8	6.9
I	Type 8 variant	15.0
150	Type 5	6.0
1	Teichoic acid[b]	ND[c]

[a] Antibodies were measured by the quantitative precipitin method (10, 11).
[b] Antibodies were prepared from rabbits immunized with noncapsulated *S. aureus* 1.
[c] ND, Not done.

more reactive with O-acetylated type 8 CPS than with de-O-acetylated CPS, induced effective phagocytosis of type 8 *S. aureus* E-2286 by PMN. Strain E-2286 reacted most strongly in the agglutination test with antiserum with *O*-acetyl polysaccharide specificity. MAb 2512 reactive with an epitope different from that of MAb 1725 was ineffective in mediating in vitro phagocytosis of type 8 *S. aureus* E-2286 by PMN. Similarly, rabbit polyclonal anti-83, which precipitates with only O-acetylated CPS, was most effective in inducing phagocytosis of type 8 organisms by PMN. Rabbit polyclonal anti-strain I serum reactive with both O-acetylated and de-O-acetylated CPS was less effective than anti-83 serum in inducing phagocytosis by PMN, despite its higher total CPS antibody level (Tables 2 and 3). These results suggest that the *O*-acetyl moiety of type 8 CPS is an immunodominant site. Antibodies directed against this immunodominant site are important in inducing phagocytosis of capsulated type 8 *S. aureus* by PMN. MAb 1716 reactivity with both O-acetylated and de-O-acetylated CPS, in contrast, was moderately effective in inducing phagocytosis by PMN and has a lower estimated antibody concentration than the other preparations.

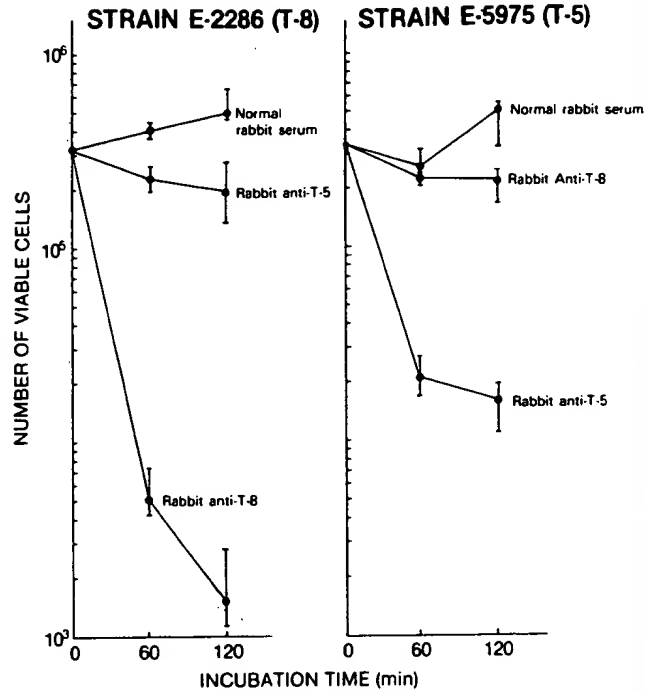

FIG. 3. Comparative effectiveness of specific type 5 and 8 antiserum in mediating phagocytosis by PMN with homologous and heterologous *S. aureus*. (Right) Strain E-2286, type 8 in the presence of type-specific antiserum. (Left) Strain E-5975, type 5 in the presence of specific antiserum.

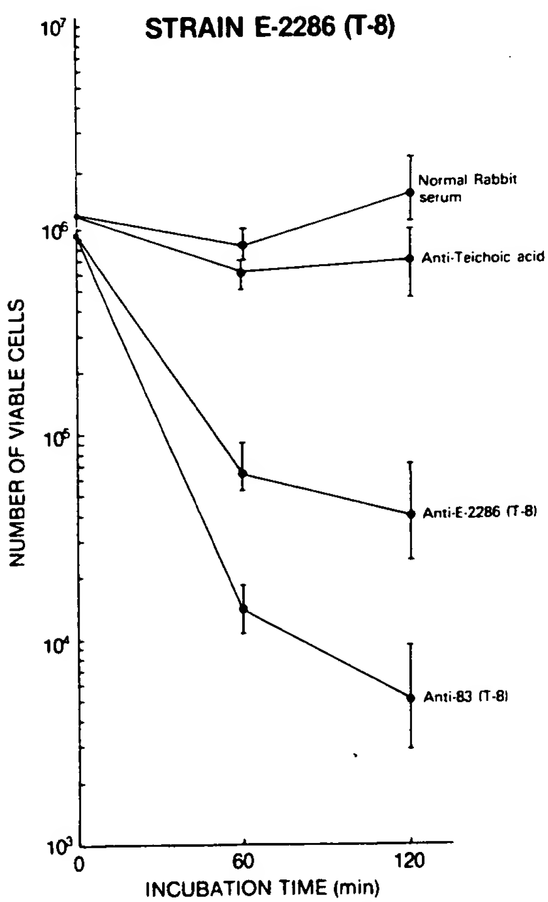

FIG. 2. Phagocytosis of type 8 strains in the presence of type-specific antisera. Anti-E-2286 and anti-83 representing the type 8 antiserum and the anti-teichoic acid serum were produced in rabbits injected with capsulated strain E-2286 (strain 83) and noncapsulated *S. aureus* (strain 1), respectively.

DISCUSSION

Phagocytosis and intracellular killing by PMN is an important host resistance mechanism against invasive infection caused by *S. aureus* (3, 26, 27). Approximately 98% of *S. aureus* blood isolates from patients are capsulated, and about 90% of the capsulated strains can be serotyped with 11 CPS typing antisera (2, 8, 11, 19, 22). Recently, the predominant type 5 and 8 CPS have been visualized on the surface of capsulated organisms by electron microscopy (8, 22). Binding studies with anti-type 5 and 8 MAbs revealed that the extent of bacterial capsulation varied; low-CPS-con-

TABLE 3. MAb concentrations

MAb	Immunizing strain	Relative titer[a]
1716	Wright (type 8)	34
1725	Wright (type 8)	59
2512	Strain I	1,600

[a] Reference MAb 1725 was defined as 59 U (23).

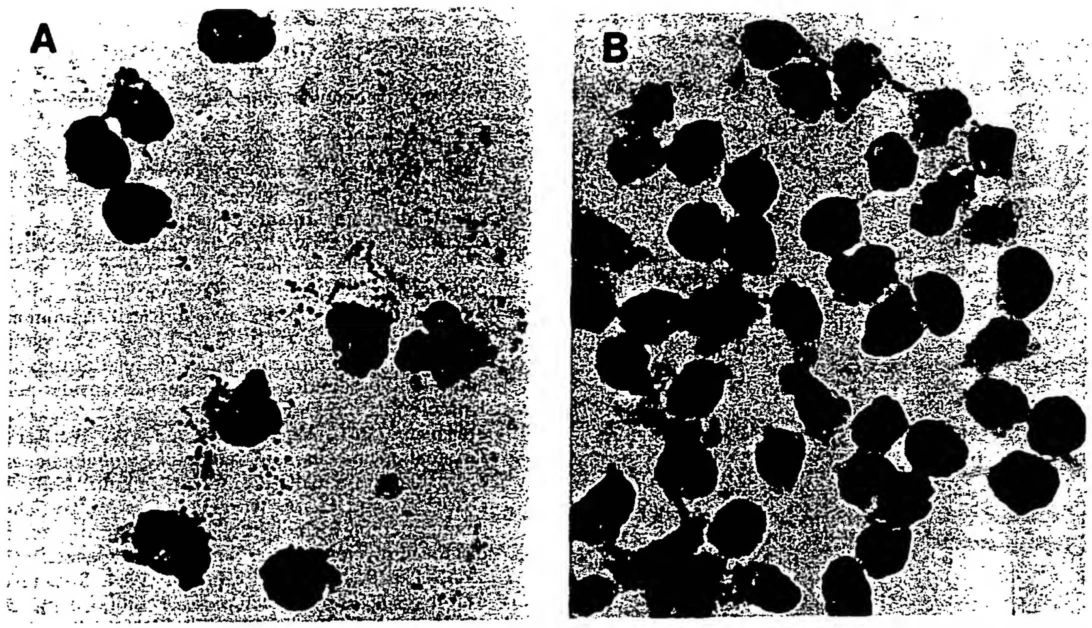

FIG. 4. Giemsa-stained smear of activated phagocytes in the presence of type 8, strain E-2286 and anti-teichoic acid antiserum prepared from rabbits injected with noncapsulated strain 1 (A) and MAb 1725 (B) (reactive with O-acetylated type 8 CPS).

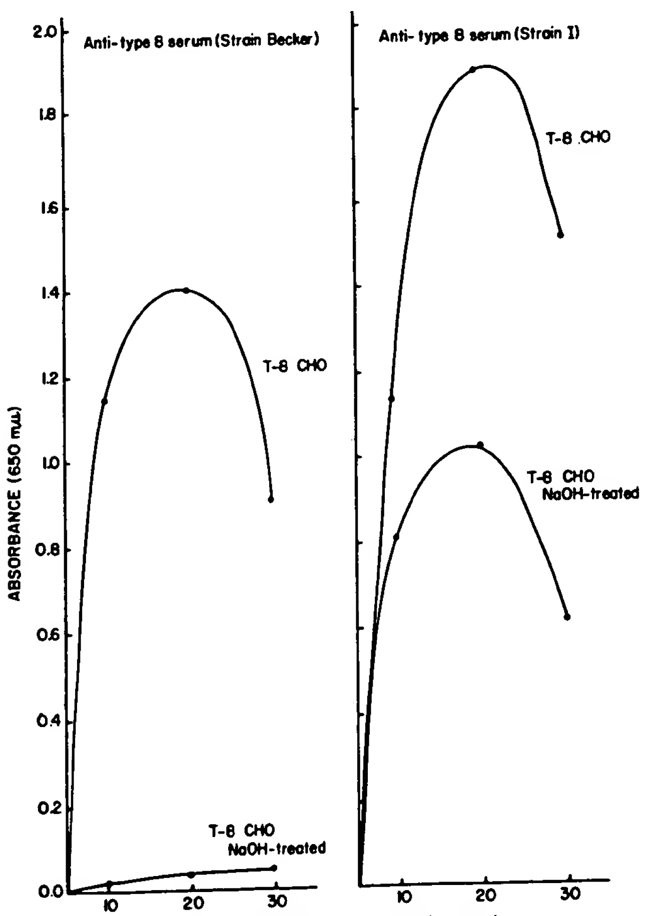

FIG. 5. Quantitative precipitin analysis of rabbit polyclonal serum elicited by multiple intravenous injections of *S. aureus* Becker (type 8 prototype) and strain 1 (type 8 variant). Equal volumes (0.1 ml) of antiserum and antigen were mixed and stored at 37°C for 1 h and overnight in the cold. The reaction mixtures were centrifuged and washed as described previously (10). The anti-capsular polysaccharide antibody concentration was determined by measuring the protein of the washed precipitates by the modified Lowry method with bovine serum albumin as a reference standard.

taining *S. aureus* bound MAb at the bacterial membrane in patches, whereas high-CPS-containing strains bound MAb in hairlike zones surrounding the cell wall (8). Capsulated strains of *S. aureus* are not agglutinated by antisera prepared against noncapsular strains and are resistant to phagocytosis by PMN (11). This antiphagocytic property of *S. aureus* CPS is similar to the virulence-promoting factors of other CPS of capsulated bacterial pathogens, including *Escherichia coli*, *Klebsiella* spp., *Haemophilus influenzae* type b, *Neisseria meningitidis*, and *Streptococcus* spp. (1, 4, 9, 18, 23). Our observations that capsulated strains of *S. aureus* are not agglutinated by teichoic acid antibodies suggest that the CPS may mask the effective opsonization by peptidoglycan antibodies, which play a major role in mediating phagocytosis of noncapsulated *S. aureus* by PMN (26, 27).

We have shown that CPS antisera induce phagocytosis of capsular type 5 and 8 *S. aureus*. The antigenic specificity of the type 8 CPS was important in inducing in vitro PMN phagocytosis of type 8 organisms. Antibodies reactive with the type 8 CPS in its O-acetylated or native configuration were effective in inducing phagocytosis of type 8 organisms by PMN; antibodies with de-O-acetylated specificity were not. Similar results on the multiplicity of epitopes on a CPS have been reported for both native and desialylated type 3 and type 1 group B hemolytic *Streptococcus* spp. (10, 18). In the case of type 1, group B *Streptococcus* spp., antibodies directed against the native sialylated CPS were more effec-

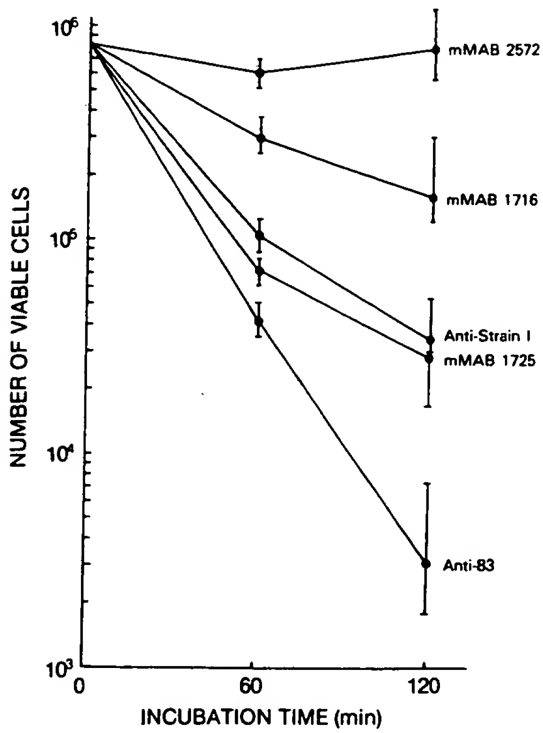

FIG. 6. The specificity of phagocytosis mediated by type 8 murine MAbs 1725, 1716, and 2512 (24, 25). Rabbit polyclonal antiserum anti-83 and anti-strain I.

tive in inducing phagocytosis of capsulated type 1 organisms than were antibodies with desialylated CPS specificity (10).

Previous studies of capsulated *S. aureus* type 1, strain M have shown that anti-CPS antibodies and complement (classical and alternative pathways) play an important role in the phagocytosis of strain M organisms by PMN (26). Verbrugh suggested two distinct mechanisms for the opsonization of capsulated *S. aureus* M. Both mechanisms require specific capsular antibodies; however, one is dependent upon the activation of complement and C3b receptor sites on PMN, and the other requires Fc receptor sites on the PMN. It was reported that high concentrations of specific capsular antibodies could enhance the in vitro phagocytosis of capsulated organisms by PMN without the addition of complement. In all of our in vitro phagocytosis studies, moderately high concentrations (1.0 mg/ml) of CPS antibodies were used in each test. We have shown that capsular antibodies induced phagocytosis of capsulated type 5 and 8 organisms by PMN without the addition of complement to the test system. The classical complement pathway is probably involved in this specific phagocytosis of *S. aureus*. Since all of the CPS antisera used in the in vitro phagocytosis test were heated at 56°C for 30 min to eliminate heat-lable components of complement, it is unlikely that the alternative pathway is a major factor in the specific phagocytosis of capsulated organisms by PMN (26). These observations are consistent with previous studies demonstrating that CPS antibodies are important in the opsonization and phagocytosis of capsulated *S. aureus* by PMN (26).

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